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Original Article

Pharmacological Evaluation of *Buchanania lanzan* Bark Against Alcohol Induced Liver Cirrhosis in Rats

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ABSTRACT

Aim: The study aimed to assess the potential of *Buchanania lanzan* bark extract in mitigating alcohol-induced liver cirrhosis effects. **Objectives:** Included evaluating antioxidant and anti-inflammatory properties, exploring interactions with liver disease-related proteins, investigating impacts on cirrhosis in rats and identifying underlying molecular pathways and targets. **Materials and Methods:** Network pharmacology study was carried out by cytoscape 3.7.2. First, bark extract was screened for *in vitro* antioxidant activity via DPPH and NO assays. Wistar rats were treated with alcohol (ethanol) 3 mL/day along with treatment standard drug silymarin and *Buchanania lanzan* bark extract (400 and 800 mg/kg) and fraction (200 mg/kg) for 21 days. Body weight, food intake, liver weight as well as liver biomarker such as ALT, AST, ALP, TP, TC, TB and hepatic antioxidant enzymes SOD, catalase, LPO, GSH were measured. **Results:** In the study we identified phytocompounds of *Buchanania lanzan* bark can modulate the effect of ethanol on AKT1, TLRs, TNF, MAPK 8 and 14 targets through mainly TNF signalling pathway, Toll-like signalling pathway and MAPK signalling pathway. *Buchanania lanzan* bark showed potent antioxidant potency on scavenging of DPPH and NO. Alcohol intoxication significantly decreases body weight, food intake and serum total protein level and increases liver weight, serum ALT, AST, ALP, TC and TB level. However, treatment with silymarin, *Buchanania lanzan* bark fraction (200 mg/kg) and extract (400 and 800 mg/kg) alters the pathological conditions induced by alcohol. **Conclusion:** The Hydroalcoholic extract and fraction of *Buchanania lanzan* bark has the potential to minimize the complications associated with ethanol induced liver cirrhosis.

Keywords: Alcoholic liver disease, Cirrhosis, *Buchanania lanzan*, Alcohol, Silymarin.

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INTRODUCTION

Alcoholic Liver Disease (ALD) poses a significant global health challenge, resulting from excessive Alcohol Consumption (ALC).[1] ALD encompasses a range of injuries from mild steatosis to advanced cirrhosis, characterized by regenerative nodules surrounded by fibrous bands in response to chronic liver injury, leading to portal hypertension and end-stage liver disease.[2] This potential damage is induced by a complex mechanism involved in alcohol metabolism, which includes various processes with alcohol dehydrogenase and aldehyde dehydrogenase generating acetaldehyde, leading to the formation of adducts with biomolecules like DNA and proteins, contributing to liver damage.[3] Acetaldehyde acts as a neoantigen, triggering inflammation by activating inflammatory cytokines such as IL-6, IL-10 and TNF-α and damaging the immun e responses. Additionally, the activation of CYP2E1 produces ROS, compromising antioxidant defences and causing hepatocyte necrosis and apoptosis due to oxidative stress.[4-6] These mechanisms play a key role in the pathophysiology of alcoholic liver disease, such as cirrhosis.[7]

Chronic Liver Diseases (CLDs) rank among the top 20 causes of disability-adjusted life years and years of life lost globally, accounting for a substantial burden.[8,9] Liver disease results in around 2 million deaths each year, with viral hepatitis, Hepatocellular Carcinoma (HCC) and cirrhosis being major contributors.[10] Alcohol is a significant factor in liver disease worldwide, with over 20 million people consuming alcohol and 75 lakhs suffering from liver disease, according to the World Health Organization (WHO).[11] Alcohol misuse leads to millions of fatalities annually, representing a significant portion of global deaths and burden.[12]

Diagnosing ALD involves examining signs of severe liver damage and alcohol use disorders.[13] Various methods such as ultrasonography, transient elastography, MRI, blood biomarker measurements and liver biopsy histology can assess the degree of alcoholic fatty liver and hepatic fibrosis.[14] In severe cases like cirrhosis or HCC, liver transplantation may be necessary,[15]

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whereas pharmacotherapy lacks specificity and is associated with side effects. In recent years, herbal treatments for hepatic problems have gained popularity worldwide due to their natural composition and fewer adverse effects compared to synthetic treatments.[16]

The present study explores the potential benefits of *Buchanania lanzan* (Chironji) bark in managing ethanol-induced cirrhosis, considering its traditional use by Ayurvedic practitioners in India and its significance in Native American folklore. Ayurvedic literature supports its use for fever, haemorrhage, thirst and dysentery.[17] The bark has been associated with various pharmacological actions, including genotoxicity and oxidative stress, while exhibiting antioxidative, anti-inflammatory, wound healing, antiulcer, antimicrobial and antibacterial properties.[18] Moreover, the bark extract has demonstrated DNA protective and cytotoxic effects in Hepatocellular carcinoma cells. The polyphenolic compounds present in the bark, such as flavonoids (quercetin, kaempferol, rutin, vincetoxicoside B), coumarins (p-coumaric acid), phenolic acids (gallic acid) and phenolic lipids (cardanol), contribute to its diverse therapeutic effects.[19-21] Notably, in rat models of cirrhosis, these compounds displayed varying degrees of antioxidant, DNA protective and anti-inflammatory effects.

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The study suggests that *Buchanania lanzan* bark may hold promising potential in the management of ethanol-induced cirrhosis, backed by its historical use in ethnomedicine and rich pharmacological profile. However, further research is required to validate its efficacy and safety in clinical settings and to understand the precise mechanisms underlying its therapeutic actions. This investigation highlights the importance of traditional knowledge in identifying potential candidates for modern medicine and emphasizes the need for further exploration of natural resources in the quest for novel treatments for liver injuries and related conditions.Top of Form

This study aimed to conduct a scientific experiment using the hydroalcoholic extract of *Buchanania lanzan* bark at different doses from a previous report to investigate its hepatoprotective activity and possible mechanism in ethanol-induced cirrhosis in male Wistar rats.

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MATERIALS AND METHODS

In vivo study

Chemicals and drugs: Standard drug: Silymarin purchased from Kshipra Biotech Pvt. Ltd., Indore. Inducing agent: Alcohol (Whiskey) purchased from local bar shops. ALT, AST, ALP, total protein, total bilirubin and total cholesterol kits were used for liver biomarker tests and procured from AGD Biomedicals Pvt. Ltd., Navi Mumbai, Maharashtra, India.

Collection and authentication of plant material: The fresh bark of *Buchanania lanzan* needed for the study was gathered from the Western Ghats region of Belgaum district, Khanapur village, Karnataka, during the monsoon period (October-November). Dr. Ajit Lingayat verified the authenticity of *Buchanania lanzan* fresh bark at Shri B. M. K. Ayurveda Mahavidyalaya in Belgaum (BMK/CRF/226/2022-2023).

Extraction of plant material

The fresh bark of *Buchanania lanzan* was cleaned with running water, shed-dried and ground into coarse powder. The hydroalcoholic extract was prepared using the cold maceration method with 70% ethanol for 7 days, followed by rotary evaporation (45°C) to obtain the extract.[22]

Fractionation of plant material

As depicted in Figure 1, polyphenolic fraction from BLBE (Buchanania Lanzan Bark extract) was prepared using the approach described by Cos *et al.*[22] Briefly, extract was separated by dichloromethane-water (1:1), DCM layer is evaporated, and purified in petroleum ether-methanol (1:1).

Determination of total phenol and flavonoid content: The amount of total phenol was calculated using the FC technique established by McDonald *et al.*[23] Using the linear regression equation, the total phenolic content was determined and reported as mg gallic acid equivalent per gram of dry extract. The total flavonoid was measured by the spectrophotometric method (760 nm) as described by Chang *et al.*[24]

In vitro antioxidant activity

DPPH assay: The DPPH assay was performed using the technique outlined by Vani *et al.*, 1997.[25] In summary, a total reaction volume of 250 µL comprises 200 µL of different concentrations of the test solution and 50 µL of 0.659 mM DPPH. The reaction mixture is incubated at 25°C for 20 min before measuring the absorbance using an ELISA at 510 nm.

NO assay: Nitric Oxide (NO) was measured using the Griess reaction. Sodium nitroprusside (5 mM) in PBS was incubated with various extract concentrations (100-1000 µg/mL) in phosphate buffer (0.25 M, pH 7.4) at 25°C for 5 hr. Control tubes with buffer but without test compounds were included. After 5 hr, 0.5 mL of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added and absorbance was measured at 546 nm.[26,27]

Ethical consideration

Adult male Wistar rats weighing 180-250 g were used in the investigation. Water and food were easily accessible during the process. These species were housed in polypropylene

cages, which were set up in thermostatically regulated rooms (25±10°C). Additionally, these cages were kept in a 12 hr cycle of day and night, with a relative humidity of approximately 60%. After a 7-day period of acclimatization to the habitat, the rats were randomly divided into our experimental groups. The experimental technique employed in the study was carried out in accordance with CPCSEA guidelines with the approval of IAEC (Institutional Animal Ethics Committee) KLE College of Pharmacy, Belagavi [Resolution No. KLECOP/CPCSEA-Reg. No.221/Po/Re/S/2000/CPCSEA].

Induction of experimental hepatotoxicity

To evaluate the effects of ethanol, employed as an inducing agent in this investigation, rats were administered 40% ethanol (2 mL/100 g, orally) for 21 days.[28]

Experimental procedure in rats

Male Wistar rats were selected and divided into 6 groups, each containing 6 animals: Group I (normal group) received normal food and water; Group II (disease control) received 40% ethanol (3 mL/day, p.o) for 21 days. Group III (standard group) received ethanol (3 mL/day) for 21 days+(100 mg/kg) of Silymarin as standard and Group IV/V received ethanol (3 mL/day) for 21 days followed by treatment with (400 and 800 mg/kg, p.o) extract of *Buchanania lanzan* bark once daily for 21 days. Group VI received ethanol (3 mL/day) +(200 mg/kg) fraction of *Buchanania lanzan* bark. After the treatment period, rats were anesthetized with diethyl ether and blood was collected via retro-orbital plexus sinus puncture. Thiopentone sodium overdose was used for euthanasia. Blood and liver samples were collected for antioxidant tests, biochemical parameters and histopathological examination.

In silico study

Identification of Bioactives

Identified bioactive compounds in the bark of *Buchanania lanzan* plant using published literature and small molecule databases such as Dr. Duke's DB and PubChem.

Retrieved chemical information such as molecular formula, molecular weight and SMILES.

Draggability Assessment

Checked the drug-like characteristics of small molecules using Lipinski's Rule of Five.

Predicted drug-likeness with DLS $p \geq 0.5$ using Molsoft.

Protein Target Identification

Predicted probable protein targets for cirrhosis and phytocompounds using GeneCards and SuperPred servers.

Retrieved Gene IDs from UniProt and visualized common targets using Venny 2.1.

Gene Enrichment and Network Analysis

Submitted Gene IDs to STRING 11.0 to identify pathways modulated by genes.

Utilized the KEGG pathway database to discover pathways associated with cirrhosis.

Built a network between compounds, targets and pathways using Cytoscape 3.6.1 with edge count as a topological parameter. Adjusted node size and color for visualization.

Estimation of parameters

Estimation of serum AST, ALT, ALP: Serum AST, ALT and ALP levels were estimated using AST, ALT and ALP kits (AGD Biomedicals Pvt. Ltd., Navi Mumbai, Maharashtra, India) to assess the extent of hepatic toxicity.

Estimation of serum total cholesterol, total bilirubin, total protein: Serum total cholesterol, total bilirubin and total protein levels were estimated using total cholesterol, total bilirubin and total protein kits (AGD Biomedicals Pvt. Ltd., Navi Mumbai, Maharashtra, India).

Biochemical assay

The level of Lipid Peroxidation (LPO), expressed in terms of Malondialdehyde (MDA), was determined using the method of Ohkawa and Yagi.[29] Glutathione (GSH) was estimated by the method of Ellman and Superoxide Dismutase (SOD) was assayed by the method of Sun and Zigman to assess oxidative stress.[30]

Histopathological examination of liver

Liver fragments from each animal were placed in 6% formalin. The liver slices were gradually dehydrated in alcohol (70%-90%) and then rinsed with large amounts of water for 12 hr to remove formalin. Similarly, alcohol was removed using chloroform, which was then replaced with paraffin. Once the shreds were poured into the L-shaped hard paraffin blocks, they were embedded. The blocks were divided into parts and placed in an oven set at 60°C for 1 hr to allow the tissue to adhere. The slices were stained with eosin and hematoxylin and under a microscope, the hepatoprotective effects of *Buchanania lanzan* bark extract and fraction were examined along with the histological alterations due to alcohol consumption.[31]

Statistical analysis

All data were expressed as mean±SEM (standard error of the mean), where $n=6$. The data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism software version 8, with $p<0.05$ regarded as statistically significant.

RESULTS

Extraction of plant material: The hydroalcoholic extract of *Buchanania lanzan* bark showed a yield of approximately 22.58% (expressed as percentage w/w).

Total phenol and flavonoid content: TPC was found to be 30.26±0.38 mg GAE/g and TFC was found to be 23.56±0.38 mg QE/g.

In vitro antioxidant assay

DPPH assay: The % inhibition of the sample (extract) was found to be 91.33% at a concentration of 800 µg/mL, along with an IC_{50} value of 109.98 µg/ µg/mL.

NO assay: The % inhibition of the sample (extract) was found to be 85.78% at a concentration of 800 µg/mL, along with an IC_{50} value of 156.65 µg/mL.

In silico study

Mining of phytocompounds and their target prediction: Through a thorough literature study and the use of the IMPPAT database, a total of 28 compounds from the bark of *Buchanania lanzan* were discovered and after screening, 12 compounds were selected for further network construction as depicted in Table 1. Canonical smiles for each phytocompound were extracted from the PubChem database. Twenty-seven protein targets associated with cirrhosis were anticipated to be modulated by the 12 phytocompounds found in *Buchanania lanzan* bark.

Mining of ethanol modulated protein molecules associated with cirrhosis: The thorough literature study and GeneCard database were used to compile a list of 66 target proteins, from which only 18 are likely involved in the pathophysiology of cirrhosis and are altered by ethanol. We examined how ethanol affected each protein target.

Mining of common targets from ethanol and phytocompounds of plant: 9 common targets of ethanol and phytocompounds from *Buchanania lanzan* bark were found using Venny 2.0.

Pathway analysis of common protein targets: as depicted in Table 2, the examination of the 9 common protein targets affected by both ethanol and the bark of *Buchanania lanzan* revealed their involvement in 14 molecular pathways linked to the development of cirrhosis.Top of Form

Gene enrichment analysis of Phytocompounds protein targets: 27 protein targets for phytocompounds were revealed to be involved in 71 molecular pathways through molecular pathway enrichment analysis. Fourteen of the 71 pathways, which are frequent routes for ethanol and phytocompounds, were strongly linked to cirrhosis and its consequences.

Protein-Protein Interaction

Top 5 genes identified which are involved in liver cirrhosis are:

AKT1 (Protein Kinase B)

Role: AKT1 is a central regulator of cell survival, metabolism and growth. It is activated in response to various signals, including growth factors and cytokines.

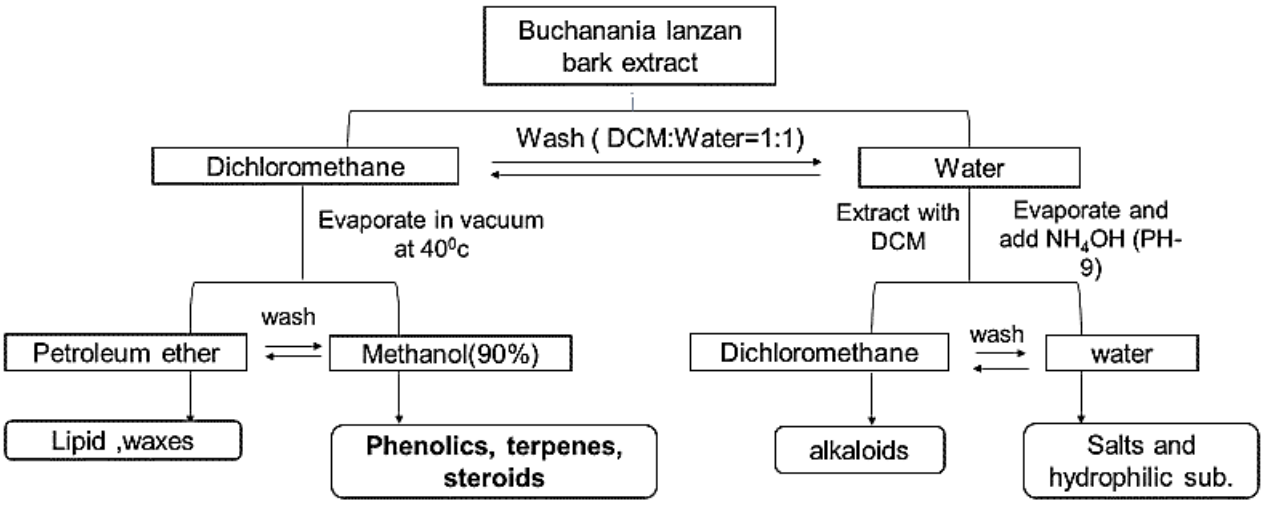


Figure 1: Fractionation of *Buchanania lanzan* bark extract.

Table 1: Molecular formula, molecular weight, no. of HBA, no. of HBD, log P value and drug likeness score of selected 12 phytocompounds.

Chemical constituents	Molecular formula	Molecular weight	No of HBA	No of HBD	Mol Log p	Drug likeness
Gallic acid	$C_7H_6O_5$	170.02	5	4	0.78	- 0.22
Quercetin	$C_{15}H_{10}O_7$	302.04	7	5	1.19	0.52
1,3,6-tri-o-galloyl-beta -D-glucose	$C_{27}H_{24}O_{18}$	636.10	18	11	0.08	0.92
Vincetoxicoside B	$C_{21}H_{20}O_{11}$	448.10	11	7	0.26	0.83
Kaempferol-7-O-glucoside	$C_{21}H_{20}O_{12}$	448.10	11	7	0.70	0.66
Ascorbic acid	$C_6H_8O_6$	176.03	6	4	1.59	0.74
Coumarins	$C_{19}H_{16}O_4$	308.10	4	1	2.32	0.59
Rutin	$C_{27}H_{30}O_{16}$	610.15	16	10	1.55	0.91
Catechin	$C_{15}H_{14}O_6$	290.08	6	5	0.53	0.64
Myricetin 3'rhamnoside-3- galactoside	$C_{27}H_{30}O_{17}$	626.15	17	11	1.94	1.04
vomicine	$C_{22}H_{24}N_2O_4$	380.17	5	1	1.55	0.98
p-coumaric acid	$C_9H_8O_3$	164.05	3	2	1.66	0.81

Table 2: Gene enrichment analysis of selected 14 pathways.

KEGG Pathway	Description Pathway	Gene Count	False Discovery Rate	Gene Codes
hsa04150	mTOR signalling pathway.	14	5.84E-06	GSK3B, PIK3CD, RPS6KA3, TNF, PIK3R1, AKT1
hsa04024	cAMP signalling pathway.	30	2.06E-15	CAMK2B, RELA, MAPK9, CHRM2, PIK3R1, PDE10.A, AKT1, GRIN2B.
hsa04151	PI3K-Akt signalling pathway.	38	8.32E-16	CHRM2, PIK3R1, AKT1, VEGFA, FGF1.
hsa04920	Adipocytokine signalling pathway.	8	0.00019	MAPK8, RELA, MAPK9, TNF, AKT1.
hsa04064	NF-kappa B signalling pathway.	14	8.91E-08	PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG2.
hsa04072	Phospholipase D signalling pathway.	17	3.44E-08	PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG3.
hsa04152	AMPK signalling pathway.	12	1.47E-05	PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG4.
hsa04620	Toll-like receptor signalling pathway.	14	8.91E-08	LCK, PARP1, PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG5.
hsa04668	TNF signalling pathway.	16	7.45E-09	PARP1, PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG6.
hsa04664	Fc epsilon RI signalling pathway.	16	1.27E-11	PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG7.
hsa04933	AGE-RAGE signalling pathway.	22	7.58E-15	PARP1, PTGS2, TLR4, SYK, RELA, IRAK4, TNF, PLCG8.
hsa04010	MAPK signalling pathway.	23	1.16E-10	DUSP3, NFKB1, FLT3, CDC25B, PDGFRA, PDGFRB.
hsa04910	Insulin signalling pathway.	13	3.18E-07	MAP2K2, PDE3B, PIK3CB, PRKACA, ACACB, PRKAA1, MTOR, PTPN1, PRKCZ.
hsa04750	IL-17 signalling pathway.	8	2.60E-05	MAP2K2, IGF1R, PIK3CB, PRKACA, ESR2, MTOR, SRC, GRB2, PIK3R1.

Interactions: AKT1 can inhibit apoptosis by phosphorylating and inactivating pro-apoptotic factors such as Bad and promoting the expression of anti-apoptotic proteins (e.g., Bcl-2).

It also modulates glucose metabolism and enhances cellular survival in conditions of oxidative stress.

TLR4 (Toll-Like Receptor 4)

Role: TLR4 is a key component of the innate immune system, recognizing pathogen-associated molecular patterns and initiating inflammatory responses.

Interactions: Ethanol can activate TLR4, leading to increased production of pro-inflammatory cytokines, such as TNF.

Inhibition of TLR4 by *Buchanania lanzan* bark extract may prevent excessive inflammation and tissue damage.

TNF (Tumor Necrosis Factor-alpha)

Role: TNF is a potent pro-inflammatory cytokine involved in systemic inflammation and the acute phase response.

Interactions: TNF activates various signaling pathways, including MAPK pathways (MAPK1 and MAPK14) and the NF-κB pathway, leading to inflammation, apoptosis and fibrosis in the liver.

High levels of TNF are associated with liver injury and the progression to cirrhosis.

MAPK1 (Mitogen-Activated Protein Kinase 1)

Role: MAPK1 (also known as ERK2) is involved in cell signaling pathways that regulate growth, proliferation and differentiation.

Interactions: MAPK1 can be activated by TNF and TLR4 signaling, linking extracellular stimuli to intracellular responses.

It plays a role in mediating the inflammatory response and cellular proliferation in the liver.

MAPK14 (p38 MAPK)

Role: MAPK14 is involved in responses to stress and inflammation, regulating cellular processes such as apoptosis and the inflammatory response.

Interactions: MAPK14 is activated by pro-inflammatory cytokines like TNF and signaling from TLR4, leading to the production of additional inflammatory mediators.

It is crucial in mediating the effects of chronic inflammation, contributing to fibrogenesis and cirrhosis in the liver.

Pathogenesis of Liver Cirrhosis

The interactions among these proteins establish a complex network that contributes to the pathogenesis of liver cirrhosis:

Inflammatory Cascade: Ethanol exposure activates TLR4, leading to TNF production and subsequent activation of MAPK pathways. This cascade results in enhanced inflammation and apoptosis of hepatocytes, driving the progression of liver damage.

Fibrogenesis: The activation of MAPK pathways (specifically MAPK1 and MAPK14) further promotes fibrogenesis through the expression of fibrogenic factors, such as **Transforming Growth Factor-beta (TGF-β):** This contributes to the accumulation of extracellular matrix components and the development of fibrosis, a hallmark of cirrhosis.

Cellular Survival vs. Death: AKT1 plays a critical role in balancing cellular survival and death. While it promotes cell survival under stress, excessive activation of inflammatory pathways (via TLR4, TNF and MAPK) can lead to hepatocyte apoptosis, creating a cycle of injury and repair that ultimately results in cirrhosis.

Potential Downstream Effects

Chronic Inflammation: Persistent activation of TLR4 and TNF leads to chronic inflammation, exacerbating liver injury and facilitating the transition from acute liver damage to cirrhosis.

Table 3: Estimation of AST (A), ALT (B) and ALP (C), TB (D), TC (E), TP (G) in the serum of rats treated with normal food and water, alcohol, silymarin, BLBE (*Buchanania lanzan* bark extract, 800, 400 mg/kg respectively) and BLBF (*Buchanania lanzan* bark fraction, 200 mg/kg).

Treatment	AST (IU/L)	ALT(IU/L)	ALP (IU/L)	TB (mg/dL)	TC (mg/dL)	TP (g/dL)
Normal	117.03±0.9	28.05±2.7	173.46±2	4.86±0.05	50.90±0.1	7.11±0.4
Disease control	231.28±0.74***	103±0.68***	332.14±1.76***	7.13±0.5***	66.22±0.2***	4.54±0.1***
Standard	143.06±0.12###	58.87±1.4###	190.5±1.6###	5.42±0.2###	52.01±0.90###	6.73±0.37###
BLBE (800 mg/kg)	189.53±0.9#@@	77.75±5.5###@@	251.75±2.6#@	6.04±0.1#@	60.34±0.6###@@@	6.47±0.1###@
BLBE (400 mg/kg)	193.68±0.6#@@@	80.62±4.7###@@@	260.46±0.8#@	6.07±0.2#@	60.61±0.1###@@@	5.78±0.2#@
BLBF (200 mg/kg)	145.53±0.1###	67.7±0.91###	204.30±1.8###	5.44±0.18###	53.47±0.28###	6.54±0.1###

Values are expressed as mean±SEM for 6 rats in each group. Where *p<0.05 when compared to normal; #p<0.05 when compared to disease control group; @p<0.05 when compared to standard silymarin considered statistically significant. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TB: Total bilirubin, TC: Total cholesterol, TP: Total protein.

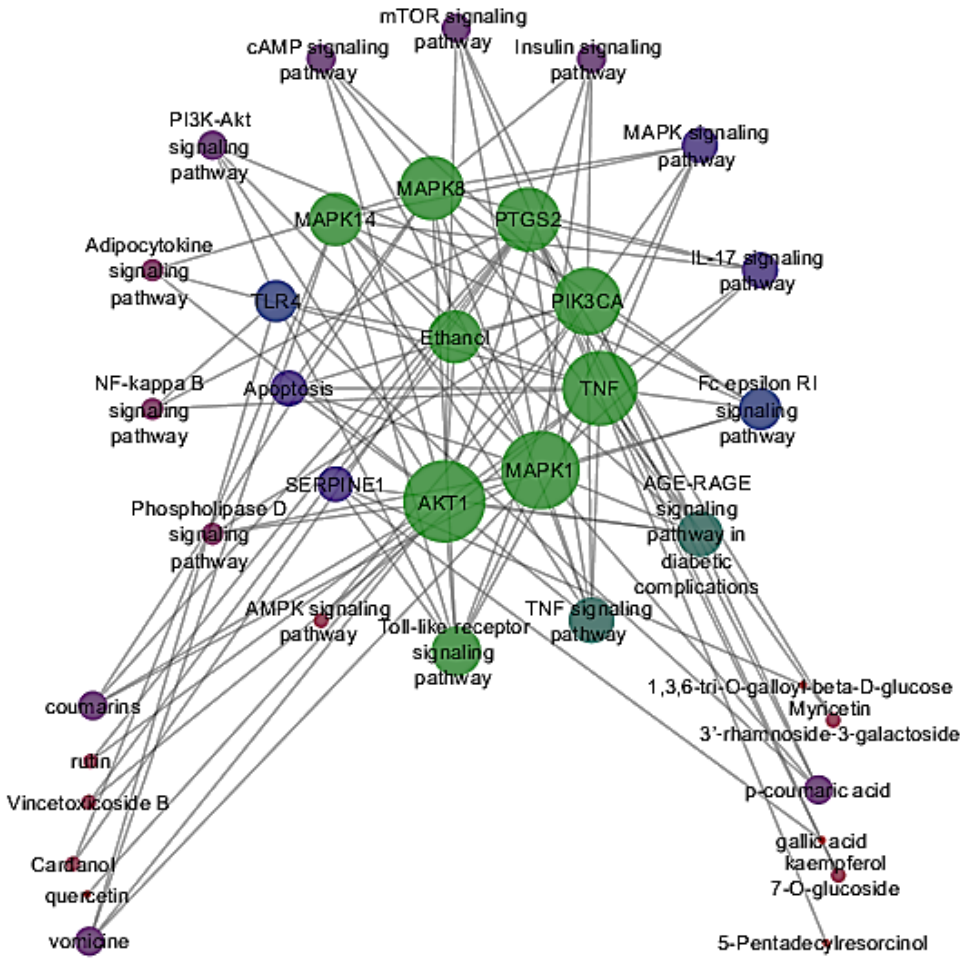


Figure 2: Protein-protein interaction.

Hepatic Stellate Cell Activation: The inflammatory environment promotes the activation of Hepatic Stellate Cells (HSCs), which contribute to the development of fibrosis.

Impaired Liver Function: As cirrhosis progresses, the loss of functional hepatocytes and the disruption of normal liver architecture result in impaired liver function, leading to complications such as portal hypertension and liver failure.

Network construction

As depicted in Figure 2, network representation of the interaction between common pathways and commonly modulated targets of ethanol and phytocompounds from *Buchanania lanzan* bark involved in the pathogenesis of cirrhosis. Middle node represent ethanol. first layer of circle surrounding the ethanol node represent protein targets. Second layer of circle represent molecular pathways modulated by targets from ethanol and phytocompounds of *Buchanania lanzan* bark.

Effect of BLBE on biochemical parameters: Table 3 and Figure 3 depicted the effect of BLBE on serum biomarker enzymes in ethanol-induced hepatotoxicity. Group II, administered ethanol, exhibited elevated levels of serum biomarkers such as AST, ALT, ALP, TB, TC and decreased TP compared to Group I (normal). Treatment with BLBE in groups IV and V slightly decreased elevated levels of AST, ALT, ALP, TB, TC and increased TP compared to Group III (standard silymarin). Group VI (BLBF) showed a significant decrease ($p<0.001$) in elevated levels of serum biomarkers AST, ALT, ALP, TB, TC and increased TP levels, like the standard silymarin group.

As depicted in Table 4 and Figure 4, BLBE improves antioxidant enzymes activity: Ethanol-administered group II reduced the levels of SOD and GSH and increased the level of LPO compared to normal group I. Treatment with BLBE in groups IV and V slightly increased the levels of SOD and GSH and decreased the elevated level of LPO. Whereas the BLBF-treated groups showed a significant ($p<0.001$) increase in GSH and SOD with a significant reduction in LPO when compared with the ethanol control group. BLBF at a dose of 200 mg/kg showed activity close to that of standard silymarin.

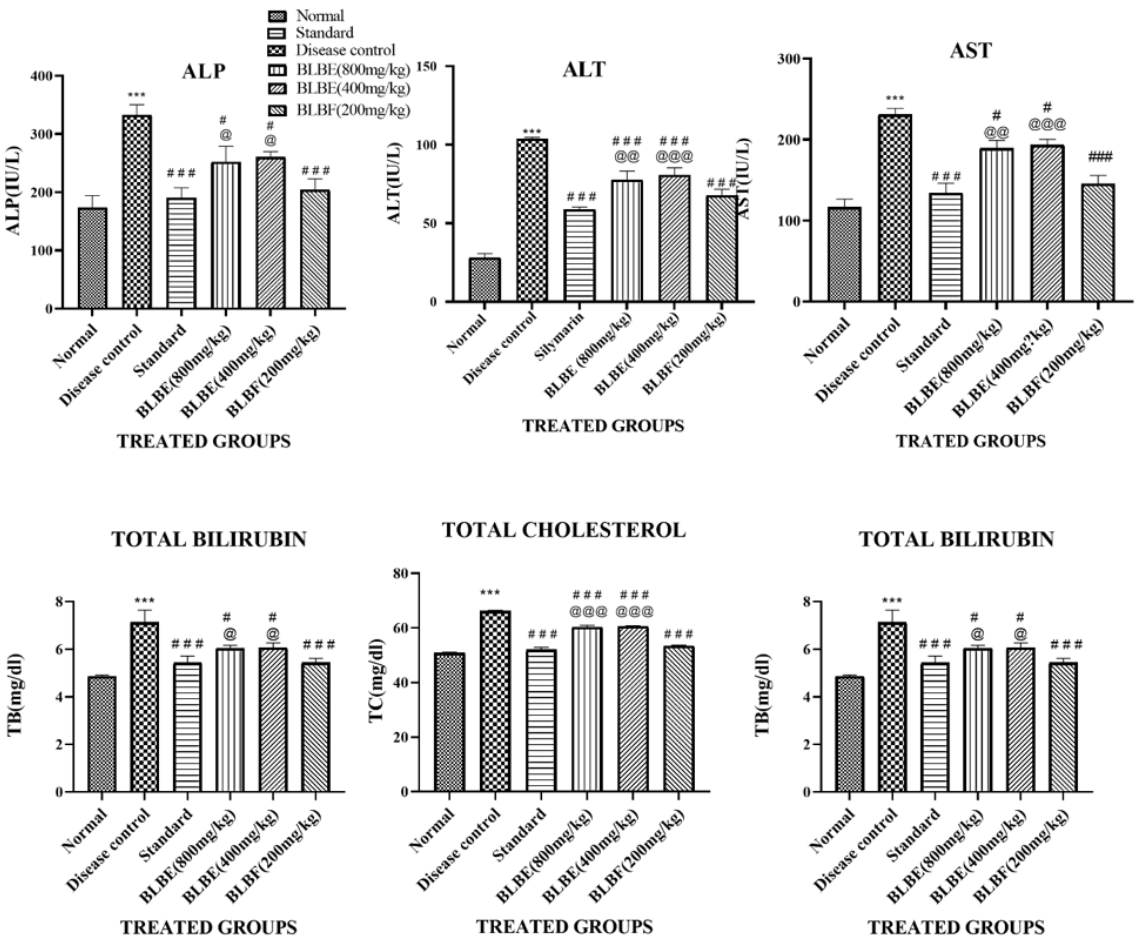


Figure 3: Estimation of AST (A), ALT (B) and ALP (C), TB (D), TC (E), TP (G) in the serum of rats treated with normal food and water, alcohol, silymarin, BLBE (*Buchanania lanzan* bark extract, 800, 400 mg/kg respectively) and BLBF (*Buchanania lanzan* bark fraction, 200 mg/kg). Values are expressed as mean±SEM for 6 rats in each group. *p<0.05 when compared to normal; #p<0.05 when compared to the disease control group; @p<0.05 when compared to standard silymarin considered statistically significant. AST: aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, TB: Total bilirubin, TC: Total cholesterol, TP: Total protein.

Histopathological studies

As depicted in Figure 5, histopathological examination of liver slices from different groups revealed various findings. The normal group (A) showed no abnormalities in hepatocytes, portal veins, sinusoids, or arterioles. In the disease control group (B), liver sections exhibited spotty necrosis, sinusoidal congestion, portal triditis, piecemeal necrosis, ballooning degeneration, small dark blue inflammatory cells, venous decongestion and bile duct proliferation. The standard group (C) displayed reduced spotty necrosis, piecemeal necrosis, ballooning degeneration, normalized portal veins with RBC rupture, venous and sinusoidal congestion compared to the disease control group. Treatment with BLBE at 800 mg/kg (D) and 400 mg/kg (E) showed slight reductions in certain abnormalities. BLBF at 200 mg/kg (G) significantly reduced multiple histopathological alterations compared to the disease control group.

DISCUSSION

Alcoholic Liver Disease (ALD) has emerged as a prominent global health concern due to the increasing prevalence of heavy alcohol consumption.[32] ALD encompasses a spectrum of liver injuries, ranging from fatty liver (steatosis) to severe cirrhosis. Despite its prevalence, a complete cure or definitive treatment for ALD remains elusive.[33] To address this issue, a multidisciplinary approach involving scientific, medical and societal efforts is necessary.[34]

The detrimental impact of excessive alcohol consumption on the liver is primarily attributed to its active metabolite, acetaldehyde. Alcohol metabolism in the liver involves key enzyme systems, namely Alcohol Dehydrogenase (ADH) and the microsomal ethanol oxidizing system (CYP2E1).[35] These enzymes produce acetaldehyde, which promotes fatty acid synthesis while inhibiting fatty acid oxidation, leading to fat accumulation in the liver and the development of fatty liver disease. Additionally, acetaldehyde forms adduct with DNA and proteins in hepatic

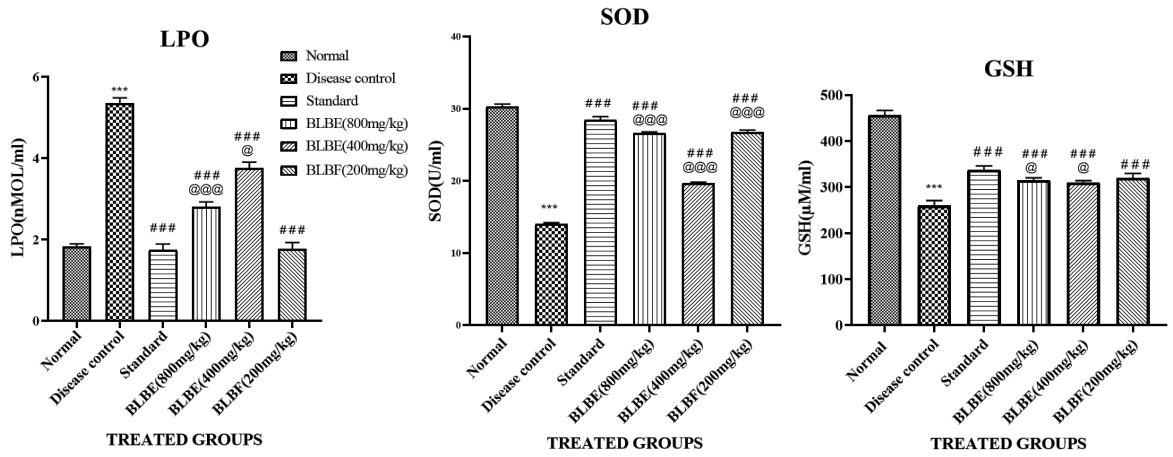
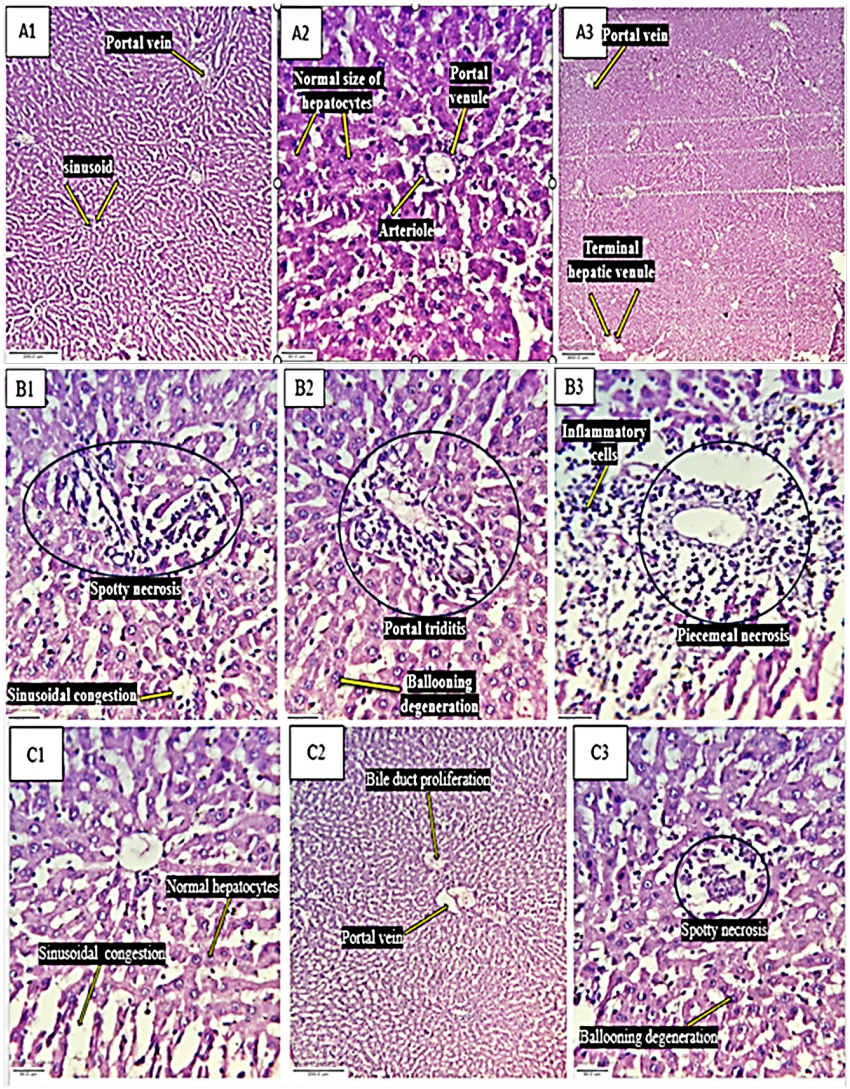


Figure 4: Estimation of SOD (A), GSH (B) and LPO (C) in the liver homogenate of rats treated with normal food and water, alcohol, silymarin, BLBE (*Buchanania lanzan* bark extract, 800, 400 mg/kg respectively) and BLBF (*Buchanania lanzan* bark fraction, 200 mg/kg). Values are expressed as mean±SEM for 6 rats in each group. *p<0.05 when compared to normal; #p<0.05 when compared to the disease control group; @p<0.05 when compared to standard silymarin considered statistically significant. SOD: Superoxide dismutase, GSH: Glutathione, LPO: Lipid peroxidation.



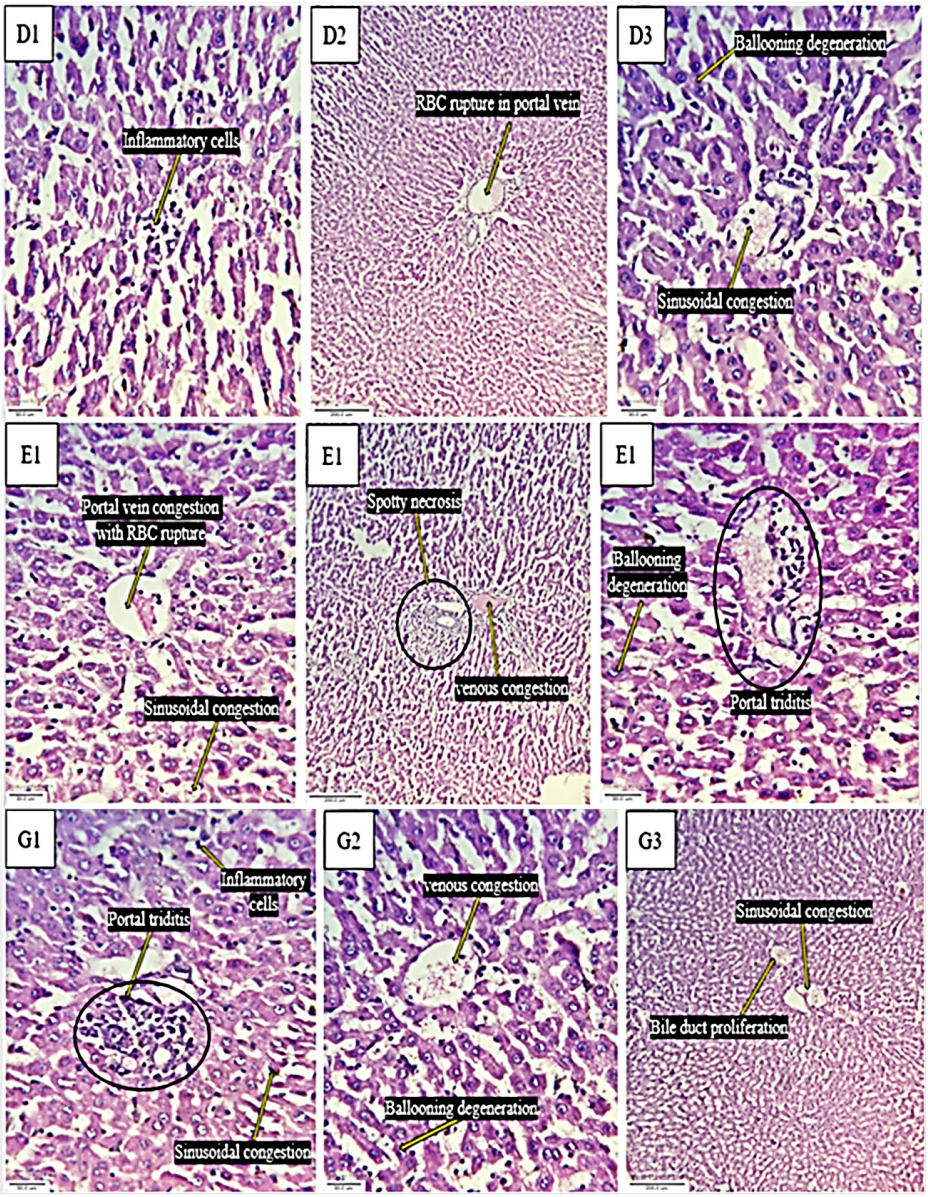


Figure 5: Histopathological changes in the liver of normal and treated animals. A1, A2, A3 - Normal group with no treatment. B1, B2, B3 - Alcohol-treated group. C1, C2, C3 - Group treated with silymarin followed by alcohol. D1, D2, D3 - Group treated with BLBE (*Buchanania lanzan* bark extract, 800 mg/kg) followed by alcohol. E1, E2, E3 - Group treated with BLBE (*Buchanania lanzan* bark extract, 400 mg/kg) followed by alcohol. G1, G2, G3 - Group treated with BLBF (*Buchanania lanzan* bark fraction, 200 mg/kg) followed by alcohol.

cells, triggering an immune response and chronic inflammation.[36] This inflammatory response aims to repair tissue damage, but an excessive response can result in scar tissue formation, disrupting liver function and causing portal hypertension. Acetaldehyde also generates harmful free radicals, causing oxidative stress and damaging lipids, proteins and DNA contributing to various liver disorders.[37]

One of the severe outcomes of ALD is alcohol-induced cirrhosis, although the precise mechanisms are not fully understood. Research into the etiology, progression and management of cirrhosis has improved patient care and life expectancy. However, current pharmacological treatments lack specificity and may lead to side effects with prolonged use, motivating researchers to explore alternative options, such as herbal formulations with potentially fewer side effects.[38]

This study aimed to investigate the potential protective effects of a hydro-alcoholic extract of *Buchanania lanzan* bark and a bark fraction against ethanol-induced liver cirrhosis in rats. Network pharmacology and gene enrichment analysis were employed to shed light on the molecular mechanisms involved in the disease and

Table 4: Estimation of SOD (A), GSH (B) and LPO (C) in the liver homogenate of rats treated with normal food and water, alcohol, silymarin, BLBE (*Buchanania lanzan* bark extract, 800, 400 mg/kg respectively) and BLBF (*Buchanania lanzan* bark fraction, 200 mg/kg).

Treatment	SOD (in U/mL)	GSH (in µM/mL)	MDA/LPO (nMol/mL)
Normal	30.28±0.3	456±0.23	1.8±0.06
Disease control	14.02±0.1***	280.31±2.6***	5.35±0.1***
Standard	28.47±0.4###	337.6±0.21###	1.74±0.1###
BLBE (800 mg/kg)	26.60±0.1###@@@	308.09±1.9###@	2.8±0.1###@@@
BLBE (400 mg/kg)	19.66±0.1###@@@	285.93±2.5###@	3.75±0.1###@
BLBF (200 mg/kg)	26.77±0.2###@@@	319.4±2.8###	1.7±0.1###

Values are expressed as mean±SEM for 6 rats in each group. *p<0.05 when compared to normal; #p<0.05 when compared to the disease control group; @p<0.05 when compared to standard silymarin considered statistically significant. SOD: Superoxide dismutase, GSH: Glutathione, LPO: Lipid peroxidation.

the modulated genes. *In silico* experiments identified polyphenolic compounds like quercetin, kaempferol, rutin, p-coumaric acid and cardanol as active components with potential therapeutic effects. These polyphenols targeted key proteins like AKT1, TLR4, TNF, MAPK1 and MAPK14, significantly modulating signalling pathways involved in cirrhosis management.[39] Excessive alcohol consumption increases endotoxin levels, activating Kupffer cells through Toll-like receptor 4 binding.[40,41] This activation triggers liver inflammation and fibrosis by inducing pro-inflammatory cytokines and profibrogenic proteins through toll-like and Akt/NFκB signalling pathways.[42] The PI3K-Akt1 pathway is crucial for acute inflammation and vascular leakage regulation.[43] MAPKs also play a pivotal role in liver inflammation control. *Buchanania lanzan* plant extract and fraction may influence these pathways, exhibiting anti-inflammatory properties that protect the liver from damage.

The antioxidant activity of *Buchanania lanzan* bark extract was evaluated through *in vitro* assays, demonstrating its potential to scavenge free radicals. Antioxidant enzymes are known to protect hepatocytes from damage. In this study, the disease control group exhibited decreased SOD and GSH levels and increased LPO levels. Treatment with *Buchanania lanzan* bark fraction and extract, along with the standard silymarin, showed significant improvements in SOD, GSH and LPO levels.

Liver function tests also provided insights. The alcohol-intoxicated group showed elevated AST, ALT, ALP, TB and TC levels, along with decreased TP levels. Conversely, groups treated with *Buchanania lanzan* bark fraction and extract, as well as standard silymarin, demonstrated improved liver function parameters.

Histopathological examination revealed distinct findings among different groups. The normal group exhibited no abnormalities, while the disease control group displayed various liver issues. The standard group showed improvements compared to the disease control. Treatment with *Buchanania lanzan* bark fraction and extract also led to improvements in histopathological alterations.

CONCLUSION

The present study demonstrated that the hydro-alcoholic extract of *Buchanania lanzan* bark and the bark fraction have potential hepatoprotective effects against alcohol-induced liver cirrhosis in rats. Molecular mechanisms and key genes were identified through network pharmacology and gene enrichment analysis. The study highlighted the potential therapeutic effects of polyphenolic compounds and their modulation of crucial signalling pathways involved in cirrhosis management. Additionally, the study emphasized the antioxidant and anti-inflammatory properties of *Buchanania lanzan* bark extract and fraction, along with their potential to improve liver function. Overall, this research contributes to the understanding of ALD and potential herbal interventions for its management. However, further research is needed to fully understand the molecular mechanisms involved and to validate these results in clinical settings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALD: Alcoholic liver disease; **ALC:** Alcohol; **IL-6,10:** Interleukin 6,10; **ROS:** Reactive oxygen species; **CLDs:** Chronic liver diseases; **HCC:** Hepatocellular carcinoma; **BLBE:** *Buchanania lanzan* bark extract; **BLBF:** *Buchanania lanzan* bark fraction; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase.

SUMMARY

Alcoholic Liver Disease (ALD) poses a significant global health challenge, ranging from mild steatosis to advanced cirrhosis. Mechanisms involving alcohol metabolism and inflammation contribute to liver damage. ALD, along with other chronic liver

diseases, ranks high among causes of disability-adjusted life years globally. Alcohol misuse leads to millions of deaths annually. Diagnosis involves assessing liver damage and alcohol use disorders through various methods. Liver transplantation may be necessary in severe cases. Herbal treatments like *Buchanania lanzan* bark show potential in managing ethanol-induced cirrhosis due to its traditional use and pharmacological properties. The bark contains polyphenolic compounds with antioxidant and anti-inflammatory effects, suggesting hepatoprotective activity. However, further research is needed to confirm its efficacy and safety in clinical settings, highlighting the importance of traditional knowledge in modern medicine's quest for novel liver disease treatments.

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